Amino Acid Composition of Six Distinct Types of β-Casein

Abstract

Aschaffenburg classified β-caseins into three groups—A, B, and C—based on their mobilities at pH 7.15 in paper electrophoresis with a citrate–phosphate buffer–containing urea. A study of the high-voltage electrophoresis diagrams of tryptic hydrolysates of a large number of β-caseins of Type A showed four distinct and different patterns. When representatives of these four types were purified by chromatography and analyzed for their amino acid composition, differences were found in histidine, proline, aspartic acid, glutamic acid, alanine, serine, valine, isoleucine, and leucine. Considering only the variation in charge-bearing groups, the four β-caseins, on the basis of five glycines per molecule, might be abbreviated as: $A^1(His_6Asp_9Glu_{40})$, $A^{2-1}(His_5Asp_{10}Glu_{39})$, $A^{2-2}(His_5Asp_{10}Glu_{38})$, and $A^{2-3}(His_5Asp_9Glu_{38})$. When the β-caseins were typed in 7% acrylamide gel at pH 9 by our usual procedure, no difference in mobility could be detected. However, if the acrylamide was increased to 10% in the gels, $A^{2-1}(His_5Asp_{10}Glu_{39})$ was faster and $A^{2-2}(His_5Asp_{10}Glu_{38})$ slower than the two other types. The majority of the β-casein[1] A samples typed agreed with $A^1(His_6Asp_9Glu_{40})$ and $A^{2-3}(His_5Asp_9Glu_{38})$ in mobility in the 10% gels. If the electrophoresis was in pH 3 acrylamide gels, the last two variants were easily distinguished, since the three $A(His_5)$ variants ran with the same mobility, but the $A(His_6)$ variant had a higher mobility. Thus, the four types can be distinguished by gel electrophoresis alone. An analysis of β-caseins B and C is included for comparison. The β-casein C was isolated by chromatography from an A/C β-casein. The A and C analyses were identical, except for addition of one histidine and one lysine and subtraction of two prolines in the β-casein C. β-Caseins B are distinguished by the presence of five arginines; A and C have only four. (B and C have both 6-histidines.)

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[1] β Casein A as used in this paper refers to β-casein typed A/A, as defined by Aschaffenburg.

Previous reports from this Laboratory on the structure of pooled β-casein described the preparation and partial structure of a large phosphopeptide (11) and an apparently homogeneous phosphorus-free peptide with a molecular weight of 6,800 (8). Later work showed this peptide to be a mixture of two peptides, one of which contained two histidine residues whereas the other contained only one. By examining a number of β-caseins from individual cows, it was discovered that some β-caseins gave both peptides and some only one. At this time Aschaffenburg published details of a paper electrophoresis method for classifying β-caseins (1) and divided them on the basis of their mobilities into A, B, and C variants. A method for classification of β-caseins by electrophoresis at pH 9 in acrylamide gels was developed in this Laboratory (12), and it was found that the pooled milk from which the histidine variants were originally obtained was of Type A. Figure 1 shows the genetic types recognizable by electrophoresis.

Following our discovery of two β-caseins Type A which contained a histidine variation

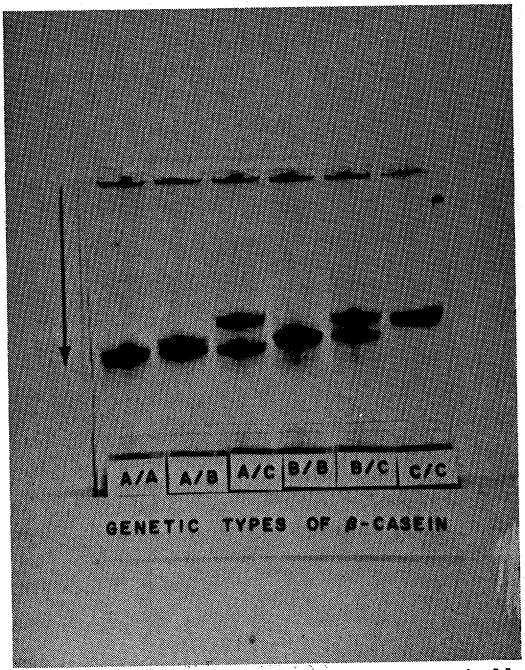


FIG. 1. Genetic types of β-casein recognizable by electrophoresis at pH 9 in 7% acrylamide gels.

(10), purified preparations of two other variants of β-casein Type A and two preparations of β-casein B and one of β-casein C have been analyzed.

Materials and Methods

Five β-caseins, three A/A and two B/B, were separated from individual milks. An A/C β-casein was used to provide an A and a C β-casein. This was obtained from the pooled milk of two cows, both typed A/C and having the same dam and sire. The caseins were precipitated at pH 4.6, washed, then given an acetic acid extraction which removed a proteolytic enzyme and a number of minor components, following the procedure of Groves et al. (4). β-Casein was isolated by following a simplified urea fractionation procedure (2) and the products dried with alcohol and ether.

The β-caseins were given a final purification by column chromatography at 3 C in 4.5 M urea, using a DEAE column and the imidazole buffers and salt gradient described by Ribadeau-Dumas (13). The A and C components of the β-casein A/C were separated by chromatography and each β-casein rechromatographed twice. To check on the purity of the preparations, they were run on acrylamide gel at pH 9.0 in 4.5 M urea (12) and evaluated by densitometry. A Canalco [2] Model E densitometer was used on sections cut from the gel. It was found necessary to extend the dyeing time to one-half hour, to saturate the peaks with dye; shorter dyeing times exaggerate the amount of purity. Integration of the densitometer peaks gave the relative amounts of impurity. No faster-moving materials were found on the gels after the first column purification. After one or two chromatographic runs the impurities were less than 1% of the main peak.

Some experiments were made with the Elphor [2] FF continuous electrophoresis apparatus (5). The faster-moving materials in β-casein could be removed easily by electrophoresis in pH 1.8, 2.5% (w/v) formic–9% acetic acid buffer, even at the rate of as much as a gram of β-casein per day. However, the slower-moving constituents could not be removed.

Gel electrophoresis at pH 9 was in a water-cooled vertical cell as described by Peterson (12). In addition, gels were prepared at pH 3 which contained 1 M acetic acid and were 4.5 molar in urea. For each 150 ml of gel solution, 1.0 ml of N,N,N′,N′, tetramethylethylene diamine and 0.3 g of ammonium persulfate were used as the catalyst. One M acetic acid was placed in the buffer vessels. The negative electrode was attached to the lower compartment of the cell and a .040-amp current run through the cell for 2 hr before putting the samples in the slots.

Phosphorus determinations were made by the method of Morrison (7). For amino acid analyses, hydrolysates of 10 mg of air-dry β-casein were made in 1 ml of 6 N glass-distilled hydrochloric acid. The tubes were frozen, evacuated, sealed off, and heated 24 or 72 hr at 110 C. Values for serine and threonine were extrapolated to zero-time. Corrections were made for moisture and ash. The ash was determined with magnesium acetate present and corrected for the phosphorus content. Three 24-hr hydrolysates and three 72-hr hydrolysates were chromatographed on a Phoenix Precision Instrument Company [2] K-5000 amino acid analyzer.

Amide nitrogen was determined by the method of Stegemen (14), which involves the diffusion of ammonia from an alkaline solution of the protein at room temperature into dilute sulfuric acid and nesslerization of the solution in the same flask. Analyses were made in triplicate at 24 and 48 hr and extrapolated to zero-time.

Hydrolysates for single-dimension trypsin fingerprints of the β-caseins were made by dissolving 25 mg of the β-casein in 10 ml of 0.2 M ammonium bicarbonate buffer at pH 8. To this, 0.25 mg of Worthington [2] trypsin (2× crystallized, salt-free) was added and the solution briefly stirred. After 1 hr the hydrolysates were heated rapidly to 90 C, then cooled, frozen, and lyophilized. The electrophoresis was conducted on a flat plate electrophoresis apparatus cooled to 5 C. The acid buffer was 2.5% formic acid–9% (w/v) acetic acid, and Whatman 3 MM paper 27 cm wide by 48 cm long was used. All the β-casein hydrolysates were applied at a line near the positive anode, then a 2,000-v power supply connected to the apparatus. After 2 hr the sheets were dried and sprayed with a ninhydrin reagent containing 0.7 g ninhydrin, 28 ml of collidine, 270 ml of acetic acid, and 700 ml of ethanol. The sheets were allowed to stand at room temperature until the colors developed. As the histidine variation is more apparent in patterns made at pH 9, a set was run in triethylamine carbonate buffer, 1.0 molar, 2 hr at 2,000 v. Cellophane barriers were used on each end of the sheet, to prevent burnout of the paper.

Tryptophane was estimated, using the molecular extinction coefficients selected by Wetlaufer

[2] It is not implied that the USDA recommends the above company or its product to the exclusion of others in the same business.

(16) for tyrosine and tryptophane. For each casein, the tyrosine was near 4.0 residues. Noltman's (9) method of hydrolysis in $Ba(OH)_2$, followed by chromatography on a 15-cm column, is being used to check the tentative figure for tryptophane.

N-Terminal group determinations were made following Fraenkel-Conrat's procedure (3). Only dinitrophenol and dinitroaniline were found in the ether-soluble fractions. DNP-arginine was identified in the chromatogram of the water-soluble DNP-amino acids. It was distinguishable from ϵ-DNP lysine, since ninhydrin did not change the color of the yellow spot, but the Sakaguchi reagent of Fraenkel-Conrat changed the yellow spot to orange.

The carboxypeptidase procedure of Kalan (6) was used to identify the C-terminal amino acid of the β-caseins.

Results and Discussion

The low histidine variants of β-casein A were discovered when a peptide from pooled β-casein A was being tested for homogeneity. It was separated into two peptides by curtain electrophoresis. Analysis for amino acid composition showed one peptide had two histidines per 6,800 MW; the other had one histidine and an added proline (10). When β-caseins from individual cows were hydrolyzed with trypsin, most of the β-caseins A gave only the 6,800 MW peptide with two histidines. A few gave both peptides.

High-voltage electrophoresis at pH 9 was used to search for differences in the mobilities of the peptides from individual β-caseins of the A type. Finally, four separate and distinct patterns were recognized. These are shown in Figure 2. Here a hydrolysate of the β-casein which gave the peptide with two histidines was applied at Locations 1 and 5. The three low-histidine β-caseins were applied at Locations 2, 3, and 4. A prominent peptide marked *X* in the figure is associated with the presence of the dihistidine peptide. It is absent in the hydrolysates of β-casein in 2, 3, and 4. When the peptide is present, the peptide at Location *Y* is colored differently by ninhydrin from the corresponding peptides in Samples 2, 3, and 4. At Location *Z* β-caseins 3 and 4 have an extra peptide not present in 1 and 2. The β-casein in Column 3 has a slow-running peptide near the origin not present in the three other β-caseins.

When β-caseins B and C had been separated and purified, trypsin hydrolysates were made, but the large 6,800 MW peptide could not be isolated from them. Electrophoresis of the trypsin hydrolysates of all the β-casein types

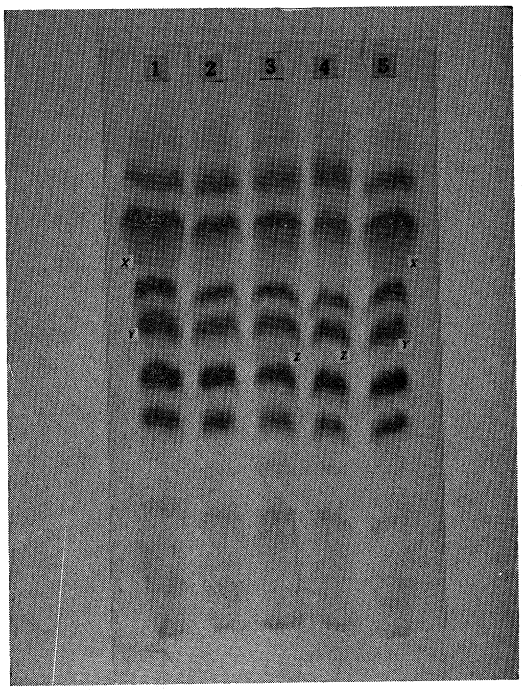


FIG. 2. High-voltage electrophoresis diagrams at pH 9 of trypsin hydrolysates of four selected types of β-casein A. Origin at top. Numbers 1 and 5 are of the same hydrolysate of β-casein A giving the dihistidine peptide; 2, 3, and 4 are β-caseins which have the monohistidine peptide.

at pH 1.8, as shown in Figure 3, demonstrates this point. Here all the Type A variants are in Columns 1 to 4, two samples of Type B β-casein in Columns 5 and 6, C is in Column 7, and pooled Type A β-casein in Column 8. The β-casein which gives the dihistidine peptide, shown in Column 1, does not have the peptide at Location *X* in Columns 2, 3, and 4. It is also missing in 5, 6, and 7. The β-caseins B are readily recognized by a prominent peptide at *Y* not present in the other β-caseins. In the hydrolysates of the β-caseins in Columns 3 and 4, a deeply colored peptide is at Location *Z*.

The evidence of the tryptic hydrolysates was that the two samples of β-casein B were identical. An apparent mobility difference between them could not be repeated. Data on the composition show a possible difference in an amide group and proline. The seven caseins which represented six distinct types were now analyzed for amino acid composition. Only 24- and 72-hr hydrolysates were made. Hydrolysis for longer periods did not result in further release of isoleucine or valine. The 24-hr figures were used for tyrosine, and the 72-hr figures for valine and isoleucine.

When results were tabulated on the basis of

TABLE 1

Amino acid composition of β-caseins
Residues of amino acid per mole (24,100)

	$A^1(His_6Asp_9Glu_{40})$	$A^{2-1}(His_5Asp_{10}Glu_{39})$	$A^{2-2}(His_5Asp_{10}Glu_{38})$	$A^{2-3}(His_5Asp_9Glu_{38})$	B-1	B-2	C
Lys	10.60 ± .05[a]	10.77 ± .14	11.05 ± .20	10.71 ± .07	11.07 ± .11	10.99 ± .23	11.64 ± .16
His	5.87 ± .08	5.10 ± .11	5.02 ± .06	4.84 ± .15	5.95 ± .06	6.03 ± .11	6.19 ± .15
NH_3	25.3	24.6	24.5	24.1	24.0	24.9	28.1
Arg	3.88 ± .04	3.98 ± .07	4.16 ± .11	3.80 ± .09	4.57 ± .17	4.97 ± .20	4.11 ± .15
Asp	9.16 ± .03	9.89 ± .16	9.76 ± .33	9.21 ± .07	9.38 ± .04	9.35 ± .08	9.22 ± .15
Thr	9.07 ± .07	8.78 ± .12	9.19 ± .20	9.54 ± .57	9.08 ± .09	9.11 ± .20	9.13 ± .09
Ser	14.98 ± .13	13.56 ± .72	14.72 ± .47	15.33 ± .28	13.89 ± .10	14.10 ± .60	14.70 ± .49
Glu	39.70 ± .27	39.40 ± .40	38.00 ± .53	38.43 ± .54	39.57 ± .69	39.55 ± .89	37.90 ± .49
Pro	33.70 ± .13	34.64 ± .04	33.34 ± .56	35.59 ± .49	33.06 ± .57	35.05 ± .88	33.70 ± .32
Gly	5.00 ± .00	5.00 ± .00	5.00 ± .00	5.00 ± .00	5.00 ± .00	5.00 ± .00	5.00 ± .00
Ala	5.01 ± .01	5.53 ± .05	5.41 ± .00	4.95 ± .03	5.37 ± .01	5.23 ± .05	5.22 ± .13
Val	18.74 ± .15	19.05 ± .64	18.32 ± .76	18.10 ± .14	18.85 ± .44	19.12 ± .54	17.90 ± .59
Meth	5.89 ± .00	5.82 ± .08	5.78 ± .08	5.79 ± .06	5.93 ± .12	5.88 ± .06	5.44 ± .13
Ileu	9.37 ± .08	9.98 ± .03	9.50 ± .43	9.28 ± .05	9.80 ± .16	9.86 ± .17	9.50 ± .50
Leu	21.34 ± .17	21.43 ± .13	21.34 ± .28	21.29 ± .16	21.26 ± .27	21.58 ± .36	21.35 ± .54
Tyr	3.88 ± .02	3.84 ± .02	4.01 ± .09	3.71 ± .13	3.94 ± .15	4.05 ± .12	3.76 ± .04
Phe	8.85 ± .03	8.71 ± .04	8.76 ± .09	8.71 ± .08	8.86 ± .09	8.85 ± .13	8.65 ± .23
Try	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Phosphorus	4.6	4.8	4.8	4.6	5.0	4.9	4.8
MW	23,836	24,006	23,471	23,635	23,920	24,113	23,602

[a] Average deviations of three 24-hr hydrolysates and three 72-hr hydrolysates.

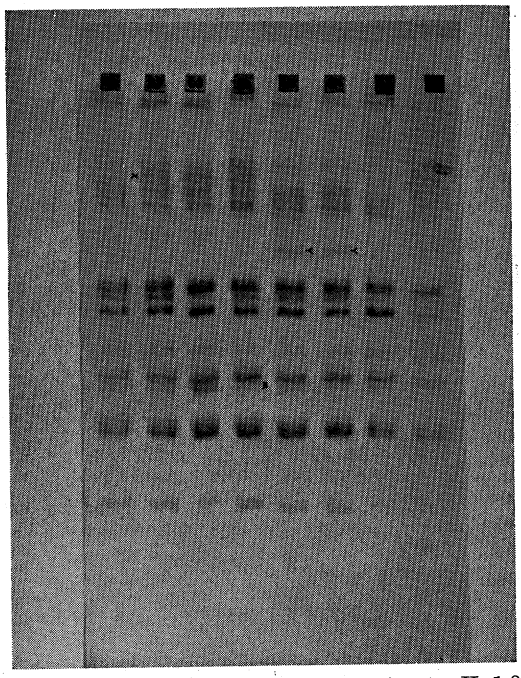


FIG. 3. High-voltage electrophoresis at pH 1.8 of trypsin hydrolysates of β-caseins. 1: β-Casein A containing dihistidine peptide; 2, 3, 4: β-caseins A containing the monohistidine peptide; 5, 6: β-caseins B; 7: β-casein C; 8: hydrolysate from pooled β-casein A. Origin at top.

24,100 MW, it was found that all the β-caseins had close to five residues of glycine per molecule. The basis of the 24,100 MW is the 24,100 ±300 figure given by Sullivan and coworkers (15), calculated from sedimentation and diffusion data. Our previous work on the phosphopeptide from β-casein which contained all the phosphorus of the β-casein (11) indicated that five phosphorus atoms were in the β-casein molecule. The phosphorus content agrees with the molecular weight of 24,100. The amino acid results were then recalculated, using a glycine value equal to five residues per mole (shown in Table 1). The β-casein which contained the dihistidine peptide is in the first column, and the three A-type β-caseins which had the monohistidine peptide are in the next three columns. The dihistidine peptide is contained in the β-caseins A which have six histidines. The variants have only five histidines. An interesting comparison is between the β-casein A in the 4th column, isolated from a β-casein A/C sample, and the β-casein C in the 7th column, isolated from the same milk. The β-casein A has two more prolines than the β-casein C, and the β-casein C has an additional lysine and an additional histidine, suggesting that lysine and histidine are replaceable by proline. The two β-caseins B are apparently identical, except for a proline difference.

End-group determinations were used as a check to see if the comparison on the basis of 24,100 molecular weight was valid. The carboxypeptidase method of Kalan (6) gives not only the C-terminal amino acid but an estimate of molecular weight as well. Data for the β-caseins in the first two columns of Table 1 are contained in Table 2. The similarity in molecular weight shows comparisons are valid. The sequence of the amino acids removed by carboxypeptidase A is valine, isoleucine, isoleucine, threonine, and serine.

If only the variations in the charged groups are considered, the four types of β-casein A may be abbreviated as: $A^1(His_6Asp_9Glu_{40})$; $A^{2-1}(His_5Asp_{10}Glu_{39})$; $A^{2-2}(His_5Asp_{10}Glu_{38})$, and $A^{2-3}(His_5Asp_9Glu_{38})$. Since the difference of 20% between six and five histidine residues should be reflected in appreciable mobility differences in gel electrophoresis at acid pH instead of at the usual pH 9, the four β-caseins of Type A and the samples of B and C were run in a 10% acrylamide gel at pH 3. The gel contained 1 M acetic acid, 4.5 M urea, and the buffer vessels contained 1 M acetic acid. Results of one such run are shown in Figure 4. Here the typing of the low histidine variants is possible. Instead of the usual mobility order at pH 9, A fastest, then B followed by C, Columns 1, 2, and 3 contain the slowest-moving β-caseins—$A^{2-1}(His_5Asp_{10}Glu_{39})$, $A^{2-3}(His_5Asp_9Glu_{38})$, and $A^{2-2}(His_5Asp_{10}Glu_{38})$. $A^1(His_6Asp_9$

TABLE 2

Release of carboxyl terminal amino acids for A-type β-caseins
Moles of amino acid released by carboxypeptidase A per 24,100 g of β-casein

	5 min	15 min	1 hr	2 hr	24 hr
$A^1(His_6Asp_9Glu_{40})$					
Valine	.212	.994	1.026	1.002	1.006
Isoleucine	.148	1.081	1.646	1.754	1.820
$A^{2-1}(His_5Asp_9Glu_{39})$					
Valine	.330	.829	.912	1.005	.965
Isoleucine	.236	.804	1.045	1.265	1.257

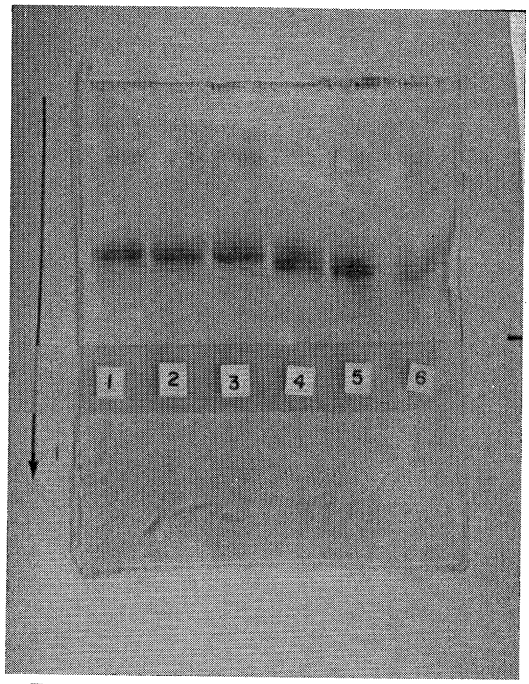


FIG. 4. Gel electrophoresis of β-caseins A, B, and C at pH 3. 1, 2, 3: β-caseins A containing the monohistidine peptide; 4: β-casein A containing the dihistidine peptide; 5: β-casein B; 6: β-casein C.

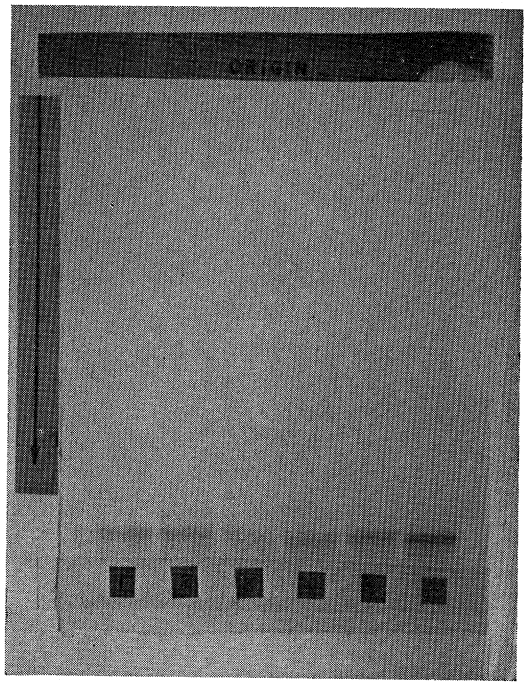


FIG. 5. Gel electrophoresis of β-caseins A at pH 9 in 7% acrylamide gel.

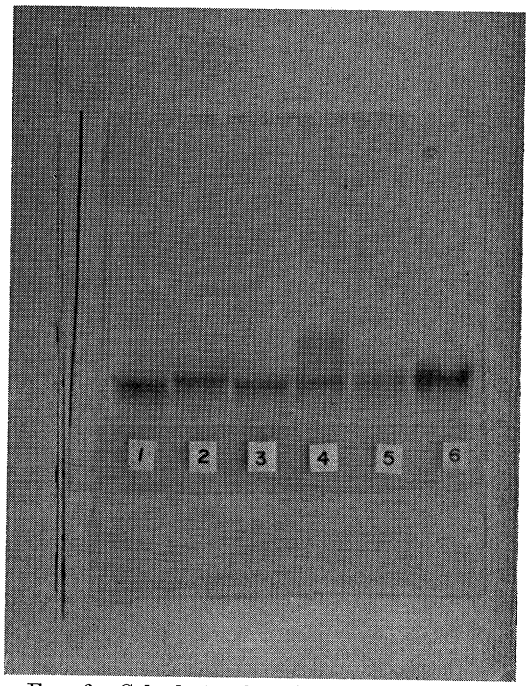


FIG. 6. Gel electrophoresis of β-caseins A at pH 9 in 10% acrylamide gels. Lines 1 and 6 are β-caseins not involved in this study. 2: β-casein; $A^{2-2}(His_5Asp_{10}Glu_{38})$; 3: $A^{2-1}(His_5Asp_{10}Glu_{39})$; 4: β-casein A/C containing $A^{2-3}(His_5Asp_9Glu_{38})$; 5: $A^1(His_6Asp_9Glu_{40})$.

Glu_{40}) is next in mobility in Slot 4; Slot 5 is β-casein B and Slot 6 is β-casein C. If the original typing of the β-casein had been done at pH 3, the variants could have been easily recognized.

The slight percentage variance in the carboxyl groups among the β-caseins A is more difficult to detect. On electrophoresis in the usual pH 9, 7% acrylamide gel, the four different types of β-casein A move to the bottom of the gel without essential difference (Figure 5). However, if the acrylamide content of the gel is raised to 10%, slight differences are apparent (Figure 6). In Figure 6 Slot 2 contained the β-casein $A^{2-2}(His_5Asp_{10}Glu_{38})$, the slowest; Slot 3 was $A^{2-1}(His_5Asp_{10}Glu_{39})$, the fastest; Slot 5 contained $A^1(His_6Asp_9Glu_{40})$; and Slot 4 had the β-casein A/C which contained $A^{2-3}(His_5Asp_9Glu_{38})$. Slots 1 and 6 contained two other β-caseins A, not a part of this study. Attempts to resolve a mixture of β-caseins A are unsuccessful.

The N-terminal amino acid for all the β-caseins was found to be arginine, and the C-terminal amino acid was valine. Molecular weights obtained by addition of residue weights ranged from 23,471 to 24,113. It is possible that some of the amino acid figures may have

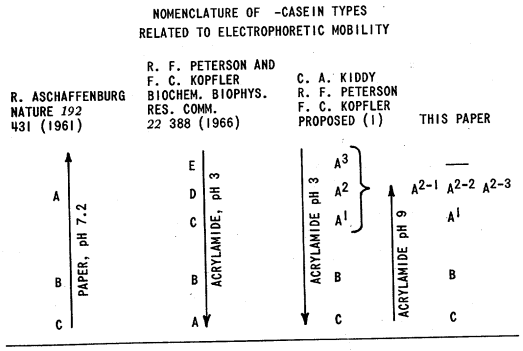
to be revised when the β-caseins are hydrolyzed to peptides and each peptide is purified and analyzed. Such work is in progress in this laboratory. After this manuscript was submitted, analyses of the genetic variants of β-casein by Pion et al. appeared in Biochemical and Biophysical Research Communications, 20: 246 (1965). In their comparison of the results between the laboratories at Jouy and Ede, for β-casein Type A, it appears that the Jouy laboratory may have analyzed a His_6/His_5 β-casein. Their results for β-caseins B and C agree with ours.

A manuscript is being prepared in collaboration with C. A. Kiddy to present results of genetic studies of the β-caseins based on the electrophoresis at pH 3. Another β-casein which moves more slowly than the His_5 variants has been found. The study has been extended to nearly 600 individual caseins.

In Table 1 the breeds were as follows: A^1, A^{2-1}, Holstein; A^{2-2}, Brown Swiss; A^{2-3} and C, Guernsey; B-1, Jersey; B-2, Brown Swiss.

TABLE 3

Clarifies the relationship of two proposed schemes for naming the new types of β-casein.



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References

(1) Aschaffenburg, R. 1961. Inherited Casein Variants in Cow's Milk. Nature, 192: 431.

(2) Aschaffenburg, R. 1963. Preparation of β-Casein by a Modified Urea Fractionation Method. J. Dairy Research, 30: 259.

(3) Fraenkel-Conrat, H., Harris, J. I., and Levy, A. L. 1954. Peptides and Proteins. Methods in Biochemical Analysis, 2: 370. Interscience Publishers, New York.

(4) Groves, M. L., McMeekin, T. L., Hipp, N. J., and Gordon, W. G. 1962. Preparation of β- and γ-Caseins by Column Chromatography. Biochim. et Biophys. Acta, 57: 197.

(5) Hannig, K. 1961. Die Trägerfreie Kontinuierliche Electrophorese und ihre Anwendung. Z. anal. Chem., 181: 244.

(6) Kalan, E. B., and Greenberg, R. 1961. The Carboxyl Terminal Amino Acids of β-Lactoglobulins A and B. Arch. Biochem. Biophys., 95: 279.

(7) Morrison, W. R. 1964. A Fast Simple and Reliable Method for the Micro-Determination of Phosphorus in Biological Materials. Anal. Biochem., 7: 218.

(8) Nauman, L. W., Peterson, R. F., and McMeekin, T. L. 1960. The Separation and Composition of Some Peptides Prepared from β-Casein by the Action of Trypsin. Abstracts of Papers, 138th Meeting American Chemical Society, 70C.

(9) Noltman, E. A., Mahowald, T. A., and Kuby, S. A. 1962. Studies on Adenosine Triphosphate Transphorylases. J. Biol. Chem., 237: 1146.

(10) Peterson, R. F., and Nauman, L. W. 1963. A New Type of Heterogeneity in β-Casein. Abstracts of Papers, 145th Meeting of the American Chemical Society, 76C.

(11) Peterson, R. F., Nauman, L. W., and McMeekin, T. L. 1958. The Separation and Amino Acid Composition of a Pure Phosphopeptone Prepared from β-Casein by the Action of Trypsin. J. Am. Chem. Soc., 80: 95.

(12) Peterson, R. F. 1963. High Resolution of Milk Proteins Obtained by Gel Electrophoresis. J. Dairy Sci., 46: 1136.

(13) Ribadeau-Dumas, B. 1961. Fractionnement de la Caseine par Chromatographie sur Colonne de Diethylaminoethylcellulose en Milieu Uere. Biochim. et Biophys. Acta, 54: 400.

(14) Stegemen, H. 1958. Eine Microbestimmung von Amid-stickstoff Speziell in Proteinen. Z. physiol. Chem., 312: 255.

(15) Sullivan, R. A., Fitzpatrick, M. M., Stanton, E. K., Annino, R., Kissel, G., and Palermiti, F. 1955. The Influence of Temperature and Electrolytes upon the Apparent Size and Shape of α- and β-Casein. Arch. Biochem. Biophys., 55: 455.

(16) Wetlaufer, D. B. 1962. Ultraviolet Spectra of Proteins and Amino Acids. Advances in Protein Chemistry. Vol. 17, p. 375. Academic Press, New York.